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Assessment of the Physiological Changes Induced by Sodium Nitrite, Annatto or Mono Sodium Glutamate in Male Albino Rats

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ABSTRACT

Background: food additives are added to most junk and fast foods, especially those for kids. Sodium nitrite is an inorganic salt with widespread applications in the food industry as a color fixative and preservative in meat and fish. Annatto extract is a natural food color obtained from the outer coatings of the seeds of the Annatto tree (*Bixa orellana L*.). Monosodium glutamate (MSG), the sodium salt of amino acid glutamate, is a food additive that popularly used all over the world as "flavor enhancer". **Aim of the work:** this study was aimed to determine the hazardous effects of sodium nitrite, annatto and monosodium glutamate on some physiological parameters in male albino rats. **Materials and methods:** this study had been done on fourty male albino rats with an average body weight 100-145 g. The animals were divided into four groups; **Group 1:** control (untreated group), **Group 2:** sodium nitrite treated group**, Group 3:** annatto treated group and **Group 4:** monosodium glutamate treated group. Blood samples were collected, sera were separated and used for estimation of some biochemical parameters (liver enzymes, kidney functions, glucose, protein profile and lipid profile) and hormonal levels [testosterone, T3 (triiodothyronine) and T4 (thyroxine**)**]. **Results:** the biochemical results showed an increase in the activities of liver enzymes [aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT)], and the levels of glucose, kidney functions (urea, and creatinine), lipid profile [total cholesterol, triglycerides, low density lipoprotein (LDL-C)] and thyroid hormones [thyroxin (T4) and triiodothyronine (T3)] in all treated groups when compared to the control group. A drop in protein profile (total protein, albumin, globulin and A/G ratio), testosterone hormone and HDL level were observed in the treated groups as compared to the control rats. **Conclusion:** it could be concluded that some food additives like sodium nitrite, annatto, and monosodium glutamate have extreme effects on liver and kidney functions, protein and lipid profiles and also on thyroid and testosterone hormones. So, it is recommended to minimize the use of these additives to protect young children and mature people from these destructive effects.

Keywords: sodium nitrite, annatto (*Bixa orellana*), monosodium glutamate (umami), liver and kidney functions.

INTRODUCTION

Humans are continuously exposed to different kinds of chemicals such as food additives. Many of these additives have been increasingly recognized as potentially hazardous to human health. Sodium nitrite is a food additive that is used, because of its role in inhibiting the growth of *Clostridium botulinum* spores in the refrigerated meats [(1)]. Meanwhile, large amounts of sodium nitrite can be toxic to animals, including humans. The cytotoxicity and detrimental effects of nitrite can be attributed to its oxidative properties [(2)]. The reactive nitrogen species that are produced by exposure to nitrite have many toxic effects including hepatotoxicity, nephrotoxicity and dysregulation of inflammatory responses and tissue injury [(3)].

Food colorants may often be considered simply cosmetic in nature, but its role is very significant. Both food quality and flavor are closely associated with color. Annatto is a natural colorant that imparts colors ranging from yellow to red due to the concentration of color compounds in the solution [(4)]. This pigment is obtained from the seed coat of the tropical shrub *Bixa orellana L*. This tree is native to tropical South America, where it has been a traditional ingredient of some foods for centuries [(5)]. Bixin and nor-bixin are the main pigments of annatto seeds that are carotenoids of huge importance in the food, pharmacological and cosmetic industries. In food industries, these natural pigments are used in cheeses, sausages, meats and candies [(6)]. Lately, FDA (Food and Drug Administration) of United States of America, classified annatto as a color additive exempt from certification that is safe for human consumption. Furthermore, many reports revealed that annatto is not carcinogenic neither maternally toxic nor embryotoxic [(7)].

Monosodium L-glutamate (MSG) is a common glutamic acid salt that contains 78% glutamic acid, 22% sodium salt and water [(8)]. MSG is the commonest food additive that has been used as a

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flavor enhancer in the home as well as in food industry since 1907 [9].

Therefore, most of the canned and fast food as flavored chips, canned soups, prepared meals, marinated meats, bottled soy or oriental sauces, freezing foods and tested tuna containing variable concentrations of MSG [10]. In animals, higher doses of MSG were confirmed to be neurotoxic as it destructs neurons in the hypothalamic nuclei through their changes in the hypothalamic-pituitary-adrenal axis (HPA) [11]. Many findings denote that unbound glutamate dissociated from MSG may possibly act on certain receptors in the central or peripheral neurons, causing many histopathological changes [12]. Moreover, the excessive MSG administration may lead to damage of liver and kidney [13].

So, the aim of this work is to investigate the hazardous effects of sodium nitrite, annatto and monosodium glutamate on some physiological parameters in male albino rats.

MATERIALS AND METHODS

fourty young male albino rats (weighing 100-145 g) were used in this study. Animals were housed in stainless steel cages, fed on rat chew and offered water *ad libidum*. The animals were divided into four equal groups (10 rats each) as follows: **The first group:** the control untreated group, **the second group:** orally administered with sodium nitrite, $NaNo_2$ (0.1 mg/kg b.wt./ day)**, the third group:** orally administrated with annatto (0.065 mg/kg b.wt./day) and **the fourth group:** orally administrated with monosodium glutamate MSG (15 mg/kg b.wt./day). Body weights were recorded every week. After 30 days of treatment, animals were weighed and then decapitated.

Blood samples were collected for biochemical parameters. Blood samples were centrifuged for 10 min. at 5000 rpm and supernatant sera were separated for analysis without storage or delay.

Biochemical Examination

In the present study total protein (TP) and albumin concentration were estimated, then serum globulin concentrations were calculated according to the formula:

Globulin (g/dl) = total protein (g/dl) –albumin (g/dl)

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) activities, Creatinine, urea, glucose concentrations as well as lipid profile that including total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were also determined. Concentrations of testosterone and thyroid hormones (T3 and T4) were measured. All parameters were estimated using **BioMerieux SA kits, France.**

The both ratios of serum albumin/ globulin and albumin/creatinine were determined. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low-density lipoprotein cholesterol) and VLDL (very low-density lipoprotein cholesterol) using the **Friedwald's** [14] and **Norbert** [15] formulas, respectively as following:

Friedewald's [14] equation: **LDL (mg/dl) = TC-{HDL + [TG/5]}.**

Norbert [15] equation: VLDL = TG/5

Statistical analysis

The results were expressed as Mean ± SEM of the mean. Data were analyzed by one way analysis of variance (ANOVA) and were performed using the Statistical Package (SPSS) program, version 20. The Bonferroni test was used as a method to compare significance between groups.

RESULTS

Body weight: animals that received sodium nitrite has a highly significant decrease in body weight ($p<0.001$), while those administrated with monosodium glutamate showed a highly significant increase in body weight ratio ($P<0.001$) and the annatto group showed insignificant changes as compared to control rats (Table 1).

Glucose level: there was a highly noticeable increase in glucose level in all the treated groups ($p<0.001$) in comparison with the control group (Table 1).

Protein profile: the present study showed that there was highly significant decrease in the total protein, albumin (except annatto) and globulin levels ($p<0.001$) in all treated groups as compared to control. Meanwhile, the treated groups recorded insignificant changes in albumin/ globulin ratio as compared to the control group (Table 2).

Liver functions: ASAT and ALAT activities revealed a highly significant increase among the treated groups in contrast to the control group ($p<0.001$) (Table 3).

Lipid profile: there was a highly significant increases in total cholesterol, triglycerides, LDL and VLDL levels ($p<0.001$) and a highly significant decrease in HDL in the treated groups ($p<0.001$) as compared to control group.

Meanwhile, annatto showed a significant increase in TC/HDL & LDL/HDL ratios ($p<0.05$), while $NaNO_2$ and MSG groups showed a highly significant increase ($p<0.001$) in both ratios in compare to control values (Table 4).

Kidney functions: there was a significant increase in creatinine value in $NaNO_2$ group ($p<0.05$), while there was no significant changes in annatto and MSG groups, whenever urea level revealed a highly significant increase in all treated groups ($p<0.001$), in comparison to the control group (Table 5).

Hormones: all treated groups revealed a highly significant decline in testosterone ($p<0.001$), while there was another highly significant increase in T3 and T4 hormones levels ($p<0.001$) as compared to the control group (Table 6).

Table (1): Percentage of body weight change and glucose level in control, NaNO2, annatto and MSG treated animals.

Parameter	Control	NaNO2	Annatto	MSG
% of body weight	35.34 ± 0.3	17.69 ± 2**	30.81 ± 2	49.31 ± 2.2**
Glucose (mg/dl)	66.6 ± 1.3	85.7 ±1.5**	72.9 ± 1.03**	77.2 ± 1.07**

Values represent mean ±SE (standard error). (P*<0.05, P**<0.001 as compared to control group).

Table (2): Serum total protein (g/dl), albumin (g/dl), globulin, albumin/globulin ratio and albumin/creatinine ratio in control, NaNO2, annatto and MSG treated animals.

Groups	Control	Sodium nitrite	Anatto	Mono sodium glutamate
Total Protein(g/dl)	6.28 ± 0.4	4.09 ± 0.2**	4.67 ± 0.19**	4.36 ± 0.18**
Albumin(g/dl)	3.86 ±0.29	2.19 ± 0.23**	3.28 ± 0.21	2.76 ± 0.19**
Globulin (g/dl)	2.43 ± 0.1	1.89 ± 0.05**	1.39 ±0.04**	1.6 ± 0.03**
Albumin/Globulin	1.58 ± 0.08	1.17 ± 0.14	2.38 ± 0.22	1.72 ± 0.14
Albumin/creatinine	7.9 ± 1.1	3.4 ± 0.7**	5.9 ± 0.7*	4.7 ± 0.6**

Values represent mean ±SE (standard error). (P*<0.05, P**<0.001 as compared to control group).

Table (3): ALAT and ASAT activities in control, NaNO2, annatto and MSG treated animals.

Groups	Control	Sodium nitrite	Anatto	Mono sodium glutamate
ALAT (U/l)	69.8 ± 0.88	88.5 ± 0.9**	81.8 ± 1.06**	84.1 ± 0.6**
ASAT (U/l)	262.7 ± 1.06	282.6 ± 1.16**	277.3 ± 0.33**	280.6 ± 1.06**

Values represent mean ±SE (standard error). (P*<0.05, P**<0.001 as compared to control group).

Table (4): Changes in total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, vLDL-C, LDL/HDL ratio and TC/HDL ratio in control, NaNO2, annatto and MSG treated animals.

Groups	Control	Sodium nitrite	Anatto	Mono sodium glutamate
Total Cholesterol(mg/dl)	**55.02 ±1.2**	**76.37 ± 1.02****	**67.44 ±1.14****	**68.92 ± 0.51****
Triglycerides(mg/dl)	**49.6 ± 0.8**	**85.05 ±0.7****	**86.75 ± 1****	**82.24 ± 1.02****
HDL-C (mg/dl)	**37.976 ± 1.2**	**19.19 ± 1.14****	**29.12± 1.1****	**24.09 ± 1.1****
LDL-C (mg/dl)	**7 ± 1.5**	**40.17 ± 3.4****	**22.17 ± 2.15****	**28.39 ± 2.7****
vLDL (mg/dl)	**9.84 ±0.37**	**17 ± 0.5****	**16.15 ± 0.5****	**16.45 ± 0.5****
LDL/HDL	**0.178 ±0.02**	**2.15 ± 0.29****	**0.77 ± 0.08***	**1.21 ± 0.18****
TC/HDL	**1.44 ± 0.02**	**4.06 ± 0.36****	**2.33 ± 0.12***	**2.91 ± 0.24****

Values represent mean ±SE (standard error). (P*<0.05, P**<0.001 as compared to control group).

Table (5): Serum creatinine and urea levels in control, NaNO2, annatto and MSG treated animals.

Groups	Control	Sodium nitrite	Anatto	Mono sodium glutamate
Creatinine(mg/l)	**0.51 ± 0.04**	**0.68 ± 0.06***	**0.56 ± 0.04**	**0.61 ± 0.04**
Urea(mg/dl)	**34.44 ± 0.6**	**44.39 ± 1.01****	**41.59 ± 0.8****	**43.45 ± 1.08****

Values represent mean ±SE (standard error). (P*<0.05, P**<0.001 as compared to control group).

Table (6): Serum Testosterone, T3 and T4 levels in control, NaNO2, annatto and MSG treated animals.

Groups	Control	Sodium nitrite	Anatto	Mono sodium glutamate
Testosterone(ng/dl)	60.6 ± 1	45.48 ± 0.9**	48.03 ± 1.2**	46.87 ± 0.76**
T3(ng/dl)	94.41 ±1.1	118.97 ± 1.7**	104.6 ± 1.5**	110 ± 1.7**
T4(ng/dl)	5.578 ± 0.19	24.19 ± 1.17**	12.54 ± 0.88**	17.48 ±1.19**

Values represent mean ±SE (standard error). (P*<0.05, P**<0.001 as compared to control group).

DISCUSSION

The goal of this study was to assess the side effects of treatment with three types of food additives (sodium nitrite, annatto, and monosodium glutamate) on some physiological parameters in male albino rats. The increase in MSG may be due to the paltability of food and disrupting the hypothalamic signaling cascade of leptin action which cause the link between monosodium glutamate and obesity and its effect on energy balance [16]. However, annatto and sodium nitrite groups recorded a significant decrease that may be related to the reduction of food utilization as reported by Grand and Butlar [17] or may be due to the increased catabolic processes in the body as reported by Til ***et al.*** [18]. Also, many researchers recorded a reduction in body weight as a result of colorants supplementation. [19]

The concentrations of total protein and albumin in the serum can be used as indicators for the state of the liver and differentiation between different types of liver damage [20].

A drop in total protein, albumin and globulin levels in NaNO2, annatto and MSG-treated groups were determined by our results. Yousef ***et al.***[21] indicated an inhibitory effect of some food additives on the biosynthesis of protein and albumin which in turn reflects that the liver is unable to perform its functions. This may be attributed to decrease protein synthesis or especially albumin through its effect on the liver by inhibiting oxidative phosphorylation process as reported by Anthony ***et al.***[22]or due to the alternation of synthetic function of the liver by MSG.

Treatment with $NaNO_2$, annatto, and MSG cause an increase in glucose levels in the blood. Similar results were obtained from Hassan ***et al.***[23] who reported an increase in glucose level of $NaNO_2$ treated groups as a result of glucogenesis, and glucose shift from tissue to blood or an impartment of glucose level mobilization. Furthermore, nitroso-compounds can alter the antioxidant system causing a disturbance in the metabolic process leading to hyperglycemia [24].

ALAT and ASAT are used as important biomarkers for the detection of the hepatotoxic effect of different materials on the liver. ALAT and ASAT activities were significant increased ($p<0.001$) in all treated groups that may be related to hepatotoxicity or destruction of the liver cells as reported by Ibrahim *et al.* [25] and Egbuonu ***et al.*** [26]. Our results are also in agreement with Poli ***et al.*** [27] who showed a dissociation of MSG to free glutamate which produces toxic ammonium ions (NH_4^+). Thus, the possible ammonium ion overload may occur as a result of an increased level of glutamate following MSG intake could damage the liver, consequently releasing the ALT enzyme that may lead to its elevation.

Data of the present work revealed a highly significant increase in serum total cholesterol, triglecerides, LDL and vLDL while HDL concentration in all treated rats showed a reduction in its level when treated with sodium nitrite, annatto or monosodium glutamate. These results run parallel with Ati ***et al.*** [28] and Hassan ***et al.*** [21]. This elevation in cholesterol may be attributed to the blockage of liver bile ducts, causing reduction or cessation of its secretion to the duodenum. Consequently, it appeared in the serum causing cholestasis [29]. Hence, the increased level of serum cholesterol noted here in rats exposed to the $NaNO_2$ could be attributed to the peroxidation of cell membrane lipids as reported by Standberg [30] and Beaupre and Schiffman [31]. The higher plasma TG could be linked to the increased number of vLDL particles that associated with visceral fat area in obese individuals as happened in MSG [32]. Low HDL-C attributed to high plasma TG that is linked to vLDL metabolism. In plasma, vLDL can exchange TG for CE (Cholesterol Ester) with HDL, a process mediated by cholesterol ester transfer protein (CETP) [33]. The exchange of lipids between these two lipoproteins leads to the production of TG-rich HDL particles [34].

Our results demonstrated an increase in serum creatinine and urea concentrations in MSG, annatto or $NaNO_2$ groups that is in agreement with

Piacenza ***et al.*** [33] and Vinodini ***et al.*** [36]**.** This may be related to changes in kidney convoluted tubules cell lining as well as in Bowman's corpuscles [37]. Furthermore, El-sheikh and Khalil [38] observed that there is an elevation in kidney functions parameters after administration of MSG, these impairments could be attributed to the changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate (GFR)**.**

There is a decrease in testosterone hormone in the treated groups as compared with control. The decrease in testosterone hormone in the MSG-treated group agrees with Burde ***et al.*** [39] and Bodnár ***et al.*** [40] that may be resulted from disruption of the hypothalamic-pituitary-testes regulatory axis that controls testosterone production by testicular Leydig cells**.** This proposition is supported by the reports of previous authors who stated that administration of monosodium glutamate destroyed neurons of the hypothalamus in rats and mice that disrupt the hypothalamic-pituitary-testes regulatory axis, and these results also agree with Ochiogu ***et al.*** [41]. Our study showed an increase in the thyroid hormones (T3 & T4) in the treated groups compared to the control. This effect could be attributed to the chemical structure of $NaNO_2$ that can compete with thyroxine – binding globulin leading to its deficiency and to hyperthyrodism by feed – back mechanism [42]. These changes in thyroid hormones could also be resulted from alteration in the pitutary – thyroid axis as a consequence of the stressing effect of the chemical component; this was in accordance with El–Saadaney [43]. Food additives can markedly alter the endocrine function of thyroid gland leading to hyper function. This might play a role in children hyperactivity probably through affecting higher centers in the brain [44].

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